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EXHIBIT A

Circumventing Tamoxifen Resistance in Breast Cancers Using Antiestrogens That Induce Unique Conformational Changes in the Estrogen Receptor[1]

Caroline E. Connor, John D. Norris, Gloria Broadwater, Timothy M. Willson, Marco M. Gottardis, Mark W. Dewhirst, and Donald P. McDonnell[2]

Departments of Pharmacology and Cancer Biology [C. E. C., J. D. N., D. P. M.], Biostatistics [G. B.], and Radiation Oncology [M. W. D.], Duke University, Durham, North Carolina 27710; GlaxoSmithKline, Research Triangle Park, North Carolina 27709 [T. M. W.]; and Bristol-Meyers Squibb, Princeton, New Jersey 08543-4000 [M. M. G.]

ABSTRACT

Tamoxifen inhibits estrogen receptor (ER) transcriptional activity by competitively inhibiting estradiol binding and inducing conformational changes in the receptor that may prevent its interaction with coactivators. In bone, the cardiovascular system, and some breast tumors, however, tamoxifen exhibits agonist activity, suggesting that the tamoxifen-ER complex is not recognized identically in all cells. We used phage display to demonstrate that the antiestrogen GW5638 induces a unique structural change in the ER. The biological significance of this conformational change was revealed in studies that demonstrated that tamoxifen-resistant breast tumor explants are not cross-resistant to GW5638. Because of these properties, this drug is currently being developed as a potential therapeutic for tamoxifen-resistant breast cancers.

INTRODUCTION

The antiestrogen tamoxifen has been used successfully for several decades as a treatment for ER[3]-positive metastatic breast cancers and as adjuvant chemotherapy (1–3). In ER-positive breast cancer cells, tamoxifen exhibits its antiproliferative, tumoristatic activities by binding to the receptor and competitively inhibiting estradiol binding (4). This simple model of tamoxifen pharmacology, however, does not explain why, in the absence of ER mutations or significant alterations in the metabolism of tamoxifen, the majority of patients with advanced disease eventually develop resistance to this drug (5–7). The model also fails to explain why a large percentage of ER-positive breast tumors exhibit *de novo* resistance to the antiestrogenic actions of tamoxifen (7). Recently, a more complex model of ER pharmacology has emerged from studies which demonstrate that the antiestrogen tamoxifen opposes estrogen action in most breast cancer cells, yet it mimics estrogen activity in the uterus, cardiovascular system, and bone (8–11). It appears, therefore, that the tamoxifen-ER complex is not recognized identically in all cells, suggesting that tamoxifen resistance and the ability of tamoxifen to function as an estrogen in some tissues may be mechanistically related. The observation that some tumors exhibit a withdrawal response upon discontinuation of tamoxifen administration supports this hypothesis (12).

Several recent studies have demonstrated that the structures of the ER-estradiol and ER-tamoxifen complexes are distinct and different from the structure of the aporeceptor (13–16). These different conformational states are thought to dictate the cellular response to agonists and antagonists by regulating the interaction of ER with coactivators and corepressors (16–18). Consequently, it has been suggested that tamoxifen resistance may be attributable to alterations in the expression level or integrity of specific receptor-associated coregulatory proteins in breast cancer cells (18). This concept was tested directly using combinatorial phage display to identify protein interaction surfaces on ERα that were exposed upon tamoxifen binding (15, 16). These studies led to the identification of peptides that interacted with ERα when activated by any ligand and others that interacted specifically with either the tamoxifen- or estradiol-occupied ER (15, 16). As expected, those peptides that interacted with the ERα-estradiol complex exclusively were able to completely block estrogen action when expressed in appropriate target cells (16). Surprisingly, peptides that interacted specifically with the tamoxifen ERα complex were able to inhibit the partial agonist activity manifested by this compound in cultured hepatocarcinoma cells, whereas they had no effect on estradiol signaling in the same system. These findings were consistent with the hypothesis that the binding of tamoxifen or estradiol to ER enables the receptor to interact with different coactivators and corepressors, and that the agonist activity of these ligands occurred by different mechanisms. This is at variance with the more popularly held models which suggest that the overexpression of coactivators, which normally interact with estradiol-activated ER, is sufficient to permit tamoxifen to exhibit partial agonist activity (17, 18). Rather, it appears that the unique conformational change within ERα that occurs upon tamoxifen binding facilitates an ectopic interaction of ERα with proteins that it would not encounter under normal physiological circumstances. This interpretation predicts that tamoxifen-refractory breast tumors should respond to antiestrogens that do not permit the presentation of surfaces on ER required for tamoxifen partial agonist activity. Evidence in support of this hypothesis was generated in the current study, which indicated: (*a*) that the antiestrogen GW5638 permits ERα or β to adopt a structure that is distinct from that observed in the presence of tamoxifen; and (*b*) that GW5638 inhibits the growth of ER-positive, tamoxifen-resistant breast tumor xenografts.

MATERIALS AND METHODS

Materials. Full-length, baculovirus-expressed recombinant ERα and ERβ were purchased from PanVera Corporation (Madison, WI). ICI 182,780 was a gift from Alan Wakeling (Zeneca Pharmaceuticals, Macclesfield, United Kingdom), raloxifene was a gift from Eric Larson (Pfizer Pharmaceuticals, Groton, CT), idoxifene was a gift from Maxine Gowan (SmithKline Beecham Pharmaceuticals, King of Prussia, PA), and GW5638 and GW7604 were synthesized by the Department of Medicinal Chemistry, Glaxo Wellcome Research and Development, Research Triangle Park, NC. 17-β-estradiol, 4-hydroxytamoxifen, and tamoxifen were purchased from Sigma Chemical Co. (St. Louis, MO). Anti-M13 antibody coupled to horseradish peroxidase was purchased from Pharmacia (Piscataway, NJ). The pMsx vector, 5× Gal4Luc3, pVP16ERα, and pVP16ERβ plasmids were gifts from Daju Fan and Ching-Yi. Chang and were created as described previously (19).

Phage Affinity Selection. Affinity selection of phage, which bound to ERα or ERβ, was performed essentially as described (19). Immulon 4 96-well plates (Dynex Technologies, Inc., Chantilly, VA) were incubated with approximately 0.25 μg (4 pmol) of ERα or ERβ, diluted in 100 μl of $NaHCO_3$ (pH 8.5) per well overnight at 4°C. The wells were blocked with 0.1% BSA and 5% milk

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[2] To whom requests for reprints should be addressed, at the Department of Pharmacology and Cancer Biology, Duke University Medical Center, Box 3813, Durham, NC 27710. Phone: (919) 684-6035; Fax: (919) 681-7139; E-mail: mcdon016@acpub.duke.edu.

[3] The abbreviations used are: ER, estrogen receptor; Gal, galactosidase; DBD, DNA-binding domain; MCF-7_{DU}/TAM, tamoxifen-refractory MCF-7 tumor.

EXHIBIT A

in $NaHCO_3$ for 1 h at room temperature and washed five times with PBST [137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4 (pH 7.3), and 0.1% Tween 20]. Next, 10 μl of the phage library diluted in 100 μl of PBST, plus 0.1% BSA and 1 μM of either GW7604 or GW5638 was put on ice for 1 h and added to the wells. The plate was then sealed and incubated, with shaking at room temperature for 5 h. Subsequent panning rounds were performed similarly but, instead, with 100 μl of phage eluate that had been amplified in *Escherichia coli* DH5αF′ cells for 5 h. Three rounds of panning were performed, and enrichment of receptor-binding phage was determined by ELISA as described below. Individual phage were plaque-purified after the second panning round, and the peptide sequences were determined by DNA sequencing.

Phage ELISA. Approximately 0.4 pmol ERα or ERβ was immobilized on the Immulon plates as described for phage affinity selection in the presence of the appropriate modulator. After blocking, 50 μl of phage from a 5-h culture grown in DH5αF′ cells were added directly to the wells, and the plate was incubated for 1 h at room temperature. Unbound phage were removed by five PBST washes, and bound phage were detected by using an anti-M13 antibody coupled to horseradish peroxidase. Assays were developed with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid; Sigma Chemical Co.) in the presence of 0.05% H_2O_2 for 10 min. Absorbance was measured at 405 nm in a microplate reader.

Cell Culture and Transient Transfection. HepG2 cells were maintained in minimal essential medium supplemented with fetal bovine serum, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (Life Technologies, Inc., Grand Island, NY). Mammalian two-hybrid assays were performed as described previously (16). Triplicate transfections contained 1000 ng of ERα-VP16 or ERβ-VP16, 1000 ng of 5× Gal4Luc3, 1000 ng of the peptide-Gal4-DBD fusion construct, and 100 ng of pCMV-βGal. Receptor modulators were added to the cells approximately 18 h before the assay.

Animals. Only female mice were used in this study. Ovariectomized athymic BALB/c or NCr nude mice were purchased from Taconic (Germantown, NY), and nonovariectomized athymic BALB/c nude mice were obtained from a colony at Duke University (Durham, NC). Mice were housed in specific pathogen-free conditions.

Establishment of MCF-7 Xenografts. The MCF-7 tumor used in these experiments was obtained from Piedmont Research Center (Morrisville, NC). The tumor was originally derived from an inoculation of 10^7 MCF-7 cells (from Piedmont Research Center, Research Triangle Park, NC) into estrogenized athymic mice as described previously (20). Estrogen stimulation was required for growth of MCF-7 tumors (data not shown). Tumor transplants were done as described previously (21).

Hormone Treatments. Pellets containing 0.72 mg of estradiol (Innovative Research of America, Sarasota, FL) were implanted s.c. on the backs of animals via trocar 1 to 3 days before tumor implantation. Pellets were replaced as needed. Tamoxifen (citrate salt) and GW5638 were administered via 0.1 ml s.c. injections at a dose of 1.0 mg/mouse, three times weekly. Compounds were suspended in corn oil (Sigma Chemical Co.) and, if necessary, sonicated briefly to aid mixing. Corn oil served as the vehicle for injections and was used to ensure that in each experiment all animals received an equal number of injections.

Development of Tamoxifen-resistant Breast Tumors. Animals bearing MCF-7 tumors were continuously exposed to daily tamoxifen injections in the presence of estrogen pellets. Tumors were passaged into new animals using 50 μl of tumor homogenate (via injections) or minced tumor pieces, implanted by trocar, approximately every 2 months. After three passages in this manner, the tamoxifen responsiveness of the tumors was assessed. It was determined that the tumors were no longer growth-inhibited by tamoxifen. Putatively, tamoxifen-resistant tumors were then transplanted into four mice which received daily tamoxifen injections; three of these tumors grew, and 6 months later, a homogenate made from one of these was implanted into eight mice. One of these tumors was then removed, minced by scalpel, and implanted into both flanks of 20 mice; 10 were supplied by Harlan Sprague Dawley (Indianapolis, IN), and 10 were from Taconic. Five animals from each vendor were treated for 5 weeks with tamoxifen injections thrice weekly, and the remaining animals received no treatment. Tumors only grew in animals receiving tamoxifen injections, suggesting that a tamoxifen-dependent tumor line had been developed (data not shown). This tumor variant was designated $MCF\text{-}7_{DU}$/TAM.

Tumor Measurements. Calipers were used twice weekly to measure bidimensional diameters of tumors. Tumor volume (in mm^3) was calculated using the formula

$$\text{Tumor volume} = [(\text{Width})^2 \times \text{Length}]/2$$

Statistical Analysis. Differences between more than two mean values were analyzed by ANOVA for normal data, and differences in more than two median values were analyzed by the Kruskal-Wallis test when the data were nonparametric. Multiple comparison tests were used to determine which groups differed in significant experiments. Experiments in which $P < 0.05$ were considered statistically significant.

RESULTS

GW5638 and Tamoxifen Are Mechanistically Distinct Antiestrogens. Previously, we identified a novel antiestrogen, GW5638, which functions as an ER agonist in bone and in the cardiovascular system but does not display uterotropic activity (22). These pharmacological characteristics indicated that, although they are structural analogues, tamoxifen and GW5638 are not identical and suggested that the conformational changes in the ER induced by these compounds might be different. To test this hypothesis, we used combinatorial phage display to identify surfaces on the ER that were exposed when the receptor was occupied by either GW5638 or its higher-affinity 4-hydroxylated analogue, GW7604, and evaluated whether or not the same conformational changes were apparent in the ER-tamoxifen complex. Specifically, we used GW7604-activated ERα or ERβ to affinity-select phage expressing receptor-interacting peptides. In all, seven libraries expressing peptides that contained one or more fixed amino acids in an otherwise random background were screened. Remarkably, all of the peptides that interacted exclusively with the ER-GW7604 complex were isolated from a library that expressed peptides containing an (X_7)Leu-X-X-Leu-Leu(X_7) motif (19; Table 1). Note the strong consensus sequence between peptides 7α-29, 7β-2, and 7β-16, where Φ represents a hydrophobic residue and Ch represents a charged residue. Using an ELISA assay, it was demonstrated that the peptides identified interact specifically with GW-activated ERα or ERβ and that no significant binding occurred in the presence of tamoxifen or any other ligand (data not shown). These findings suggested that GW5638 and GW7604 induce similar alterations in ER (α or β) structure and that these ligand-bound receptor complexes are distinct from those formed upon tamoxifen binding. This was confirmed in cells using a two-hybrid assay (16) in which sequences corresponding to the GW-specific peptides were expressed in mammalian cells as GAL4-DBD fusion proteins. The ability of these peptides to recruit an ER-VP16 fusion protein in the presence of different ligands was assessed using a GAL4-responsive reporter gene. The results of this analysis, shown in Fig. 1, indicate that within the context of intact cells, the GW-ER complexes can be distinguished from those formed in the presence of other ER agonists and antagonists. Thus, we proceeded to examine the pharmacological consequences of these distinct conformational states of the receptor in models of ER-dependent breast cancer.

GW5638 Inhibits the Growth of Estrogen-dependent Tumors in Athymic Mice. It is likely that the antimitotic activity of tamoxifen is a reflection of its ability to competitively inhibit the actions of

Table 1 *Sequences of peptides that interact with ERα or ERβ in the presence of GW5638 or GW7604*

Peptide	Sequence
7α-29	Q P L I A K W L P Y L L E E T V L V G
7β-2	S W L I D A H L A P L L F N N T L G S
7β-16	H F L I N Q H L Y K L L Q D T D I V V
Consensus	ϕ L I X X(Ch) L X X L L X X X X(L/I)
7α-9	N A L I V P H L Y E L L K R E W Q S V
7β-19	P W L D P E K L A R L L E V P V Q D F

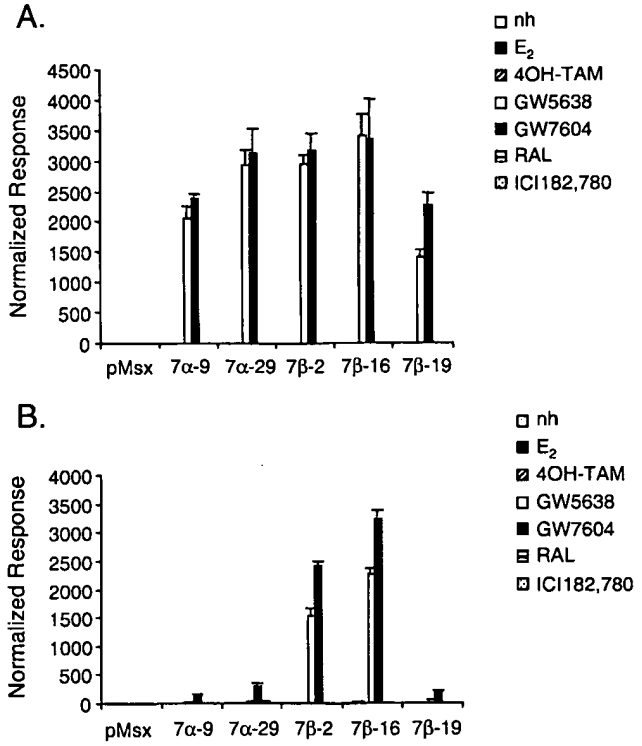

Fig. 1. ERα and ERβ adopt unique conformations upon binding the antiestrogen GW5638. A series of peptides that bound to surfaces exposed on GW5638-activated ERα (*A*) or ERβ (*B*) were identified using combinatorial phage display. The coding sequence of each peptide was fused to the DBD of the yeast transcription factor Gal4, as described previously (19). HepG2 cells were transiently transfected for 5 h with expression vectors for ER-VP16 and the peptide-Gal4 fusion proteins. A luciferase reporter construct under the control of five copies of a Gal4 upstream enhancer element and a pCMV-β-galactosidase (β-Gal) vector were cotransfected to normalize for transfection efficiency. pMsx represents the Gal4-DBD alone, which served as a negative control. Cells were treated with various ligands and assayed for luciferase and β-Gal activity. Modulators were used at 100 nM with the exception of GW5638, which was used at 1 μM because of its lower affinity for the receptor. Normalized response was obtained by dividing the luciferase activity by the β-Gal activity. Transfections were performed in triplicate, and data represent mean values. *Bars*, SD. The results shown are representative of multiple experiments performed under the same conditions. *nh*, no hormone; E_2, 17-β-estradiol; *4OH-TAM*, 4-hydroxy tamoxifen; and *RAL*, raloxifene.

estradiol in ER-positive cells. However, it has also been suggested that tamoxifen possesses additional activities that are required for its chemotherapeutic abilities (23, 24). Thus, because GW5638 is mechanistically distinguishable from tamoxifen, it was important to determine whether this compound differs from tamoxifen in its ability to inhibit the growth of ER-positive breast tumor implants in athymic nude mice. For this study, breast tumors derived from ER-positive MCF-7 breast cancer cells were implanted into the flanks of ovariectomized athymic nude mice (25). The estrogen-dependent tumors were maintained by implanting into each mouse a slow-release pellet containing 17-β-estradiol. Once tumors were palpable, mice received s.c. injections of tamoxifen, GW5638, or vehicle three times weekly. At equal doses, 1.0 mg/injection, GW5638 and tamoxifen inhibited estrogen-dependent tumor growth (Fig. 2, $P < 0.05$). Both the tamoxifen- and GW5638-treated groups differed significantly from the control group. These results indicate that although GW5638 is pharmacologically and mechanistically distinct from tamoxifen, it retains the ability to inhibit the growth of ER-positive, estrogen-sensitive breast tumor explants.

GW5638 Inhibits the Growth of Tamoxifen-refractory Breast Tumor Explants. Our data, combined with those of others, support a definitive link between the structure of an ER-ligand complex and its biological activity (13, 15–17, 26, 27). Because our peptide binding studies indicated that the GW5638-ER and tamoxifen-ER complexes are structurally distinguishable, we considered it likely that breast cancer tumors that are resistant to the antiestrogenic actions of tamoxifen would not be cross-resistant to GW5638. To test this hypothesis, we took advantage of the observation that continuous tamoxifen administration to mice bearing MCF-7 tumors leads to the development of tumor lines that are no longer growth-inhibited by tamoxifen (20). We created a subline of such tumors, designated MCF-7_{DU}/TAM, whose growth can be stimulated by tamoxifen, and we subsequently used this model system to evaluate GW5638 further. The generation of tamoxifen-resistant tumors, which occurred over a 2-year period, involved continuous dosing with tamoxifen and sequential passages of the tumors in athymic nude mice. The growth characteristics, in the presence of tamoxifen, of one of the tumor lines derived in this manner is shown in Fig. 3. For this analysis, ovariectomized, athymic nude mice (obtained from two different vendors) were implanted with putative tamoxifen-resistant tumors and treated either with or without tamoxifen for 5 weeks; tumors grew only in those animals treated with tamoxifen. The MCF-7_{DU}/TAM tumors differ from the MCF-7 line in that they do not require estrogen supplementation for growth, and they exhibit a slower growth rate than the parental line. The properties of these tumors were not

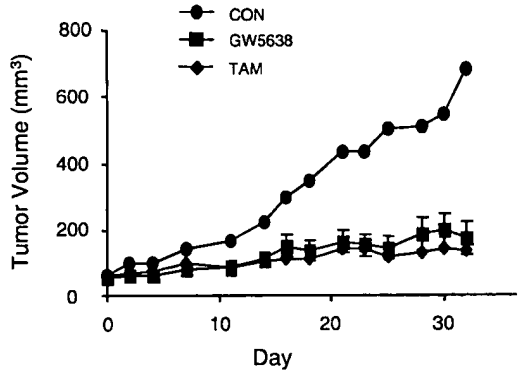

Fig. 2. GW5638 inhibits breast cancer tumors in athymic nude mice. Estrogen-dependent MCF-7 tumors were implanted into estrogenized, ovariectomized mice. After tumors were established, mice were randomized and given vehicle (●), tamoxifen (◆), or GW5638 (■) three times weekly. Day 0 represents the first day of treatment, approximately 4 weeks after tumor implantation. Data are expressed as mean tumor volumes (n = 8–9 mice/group). Animals that died during the experiment (five total) were included for the calculation of mean tumor volumes until their death.

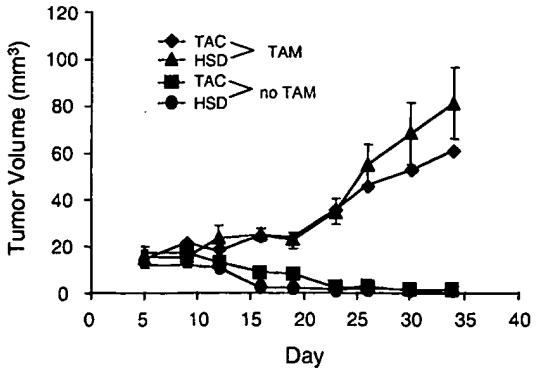

Fig. 3. MCF-7_{Du}/TAM tumors are tamoxifen dependent. Putative tamoxifen-resistant tumors were implanted into both flanks of 20 estrogenized, ovariectomized, athymic nude mice. Because of the extensive amount of surgery required to prepare these mice for our studies, and to account for any confounding influences that this may have on the results, we chose to use 10 animals from each of two vendors for these studies (*HSD*, Harlan Sprague Dawley; *TAC*, Taconic). One-half of the animals in each group were left untreated, whereas one-half received tamoxifen (*TAM*) three times a week. Tamoxifen was administered as a 1.0-mg injection in 0.1 ml of corn oil. Day 0 corresponds to the day of tumor implantation; treatment began immediately. Data are expressed as mean tumor volume. *Bars*, SE.

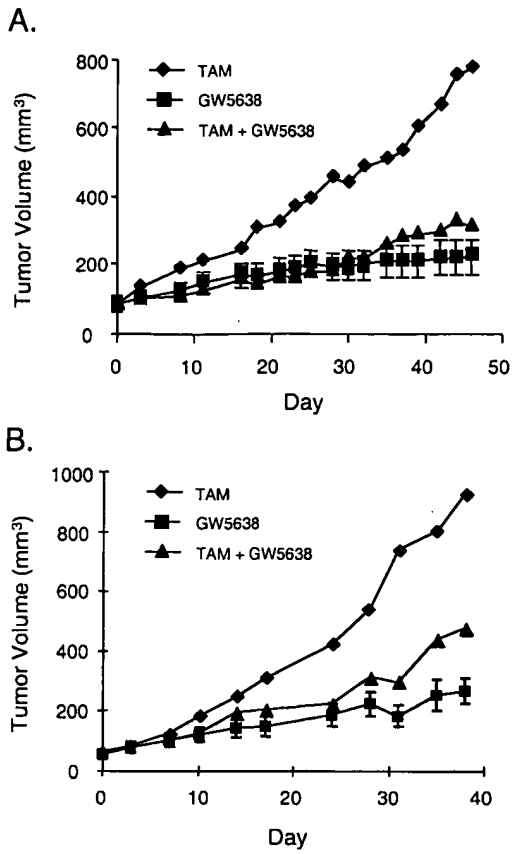

Fig. 4. Inhibition of tamoxifen-refractory tumors by GW5638. MCF-7_{DU}/TAM tumors were implanted into athymic ovariectomized mice; tamoxifen was administered to promote tumor growth (*A*). After tumors were measurable, animals were randomized by tumor volume into treatment groups as follows: tamoxifen (♦), GW5638 (■), and tamoxifen + GW5638 (▲). Data are expressed as mean tumor volumes; n = 8–10 mice/group. Tumor measurements of two animals that died randomly during the study were included in the mean volumes until the animals died. *B*, same as in *A* except that this experiment was performed in ovary-intact mice. One animal died during the study.

compared directly with tumors produced by other laboratories in a similar manner. However, they appear to exhibit similar phenotypic characteristics (25, 28).

MCF-7_{DU}/TAM tumors were implanted into ovariectomized, athymic mice. Tamoxifen was administered to all animals to promote tumor growth. Once tumors were measurable, mice were randomized into three treatment groups: tamoxifen, GW5638, or both tamoxifen and GW5638. As demonstrated in Fig. 4*A*, tumoristatic doses of GW5638 inhibited MCF-7_{DU}/TAM tumors in both the presence and absence of tamoxifen ($P < 0.05$). An analogous experiment was performed in ovary-intact mice, under conditions in which estrogen is present. Tamoxifen was initially given to all animals until tumors were visible; the mice were then randomized into three treatment groups, as above. The results of this analysis, shown in Fig. 4*B*, confirm the ability of GW5638 to inhibit the growth of tamoxifen-resistant tumors and demonstrate that this inhibitory activity occurs even in an ovary-intact animal ($P < 0.05$). Cumulatively, these results suggest that GW5638 may have clinical utility as a chemotherapeutic agent for the treatment of both tamoxifen-naive and tamoxifen-resistant, ER-positive breast tumors.

DISCUSSION

One of the most enigmatic problems in ER pharmacology is the process by which breast cancer cells switch from recognizing tamoxifen as an antiestrogen to responding to it as an estrogen (6). This change in tamoxifen pharmacology does not appear to result from alterations in the metabolism of the drug or to relate to changes in the expression level or integrity of the receptor protein. Instead, it relates most likely to changes in the processes that enable cells to distinguish between agonist- and antagonist-activated ERs (7, 29). It is now well established that the relative agonist/antagonist activity of tamoxifen can differ from cell to cell (30, 31). For instance, whereas tamoxifen functions as a partial agonist in the uterus, it manifests antagonist activity in most breast cancer cells (2, 9). On the basis of these and similar findings, we hypothesized that under the selective pressure of tamoxifen administration, breast cancer cells undergo an adaptive change that enables them to recognize tamoxifen as an agonist (32). Thus, tamoxifen resistance may reflect the ability of cells to facilitate partial agonist activity. The observation that most tamoxifen-resistant breast tumor explants are cross-resistant to other ER partial agonists, such as toremifene and idoxifene, supports the proposed relationship between resistance and ERα partial agonist activity.[4] Recently, we have identified a surface on ERα that is exposed only when the receptor is bound to tamoxifen; we have also demonstrated that the introduction into cells of peptides that bind to this surface inhibits the partial agonist activity of tamoxifen (16). This implies that in the presence of tamoxifen, ERα interacts in an ectopic manner with a factor(s) that enables this compound to manifest partial agonist activity. Formal proof of this hypothesis awaits the identification of proteins that interact with the tamoxifen-specific surfaces and whose over- or under-expression can alter tamoxifen pharmacology. One protein that may have this unique property has been reported previously (16, 33), and the significance of this observation is currently under investigation.

In this study we have used a series of specific peptide probes to show that both GW5638 and GW7604 induce a conformational change within ERα that is distinct from that induced by tamoxifen or any other ER antagonist. The significance of these conformational changes was highlighted by demonstrating that GW5638 is capable of inhibiting the growth of tamoxifen-resistant breast tumor explants in athymic nude mice. Previously, the only antiestrogen that has been shown to be able to inhibit the growth of tamoxifen-resistant tumors is ICI182,780 (34, 35). However, it now appears that this compound functions as an antiestrogen in these tumors by inducing receptor degradation (36), potentially limiting its therapeutic utility. The selective estrogen receptor modulator raloxifene, which displays minimal partial agonist activity in the reproductive systems of rodents and humans, was not found to be an effective second-line therapy for tamoxifen-refractory breast tumors (37–39). At first glance, this appears to rule out the link between resistance and ER-partial agonist activity. However, the failure of raloxifene in this clinical setting may have more to do with its poor pharmacokinetic properties than with its molecular mechanism of action (39). Regardless of whether or not the proposed model is correct, it is clear that the growth of tamoxifen-resistant tumors can be inhibited by GW5638. To our knowledge, ICI182,780 and GW5638 are the only antiestrogens to have demonstrated this activity (40). On the basis of this finding, GW5638 will be introduced into the clinic (under the name DPC-974) for evaluation as a treatment for tamoxifen-resistant and late-stage metastatic breast cancers.

Phage display was used in this study to map the potential protein interaction surfaces on the ER that are presented upon tamoxifen or GW5638 binding. As a result, a series of ligand-specific ER peptides were identified. It was remarkable, however, that the only peptides

[4] V. Craig Jordan, personal communication.

identified came from a phage library that expressed Leu XX leu leu (LXXLL)-containing peptides. This is particularly interesting because the LXXLL motif has been shown to be present in a large number of different transcriptional coactivators, enabling them to interact with the AF-2 domain of agonist-activated ER (14, 19, 41, 42). It was not anticipated, therefore, that any protein or peptide that contained an LXXLL motif would be capable of interacting with antagonist-activated ER. Interestingly, the LXXLL-containing peptides found in our screens did not require an intact AF-2 domain, and deletion of the entire ERα helix12 did not influence their receptor-binding characteristics. These data raise the possibility that there are other domains on ERα to which LXXLL-interacting coactivators can bind.

Our data support a relationship between the partial agonist activity of tamoxifen and the development of resistance. However, what has not been resolved by these, or other, studies is how compounds like GW5638, tamoxifen, and raloxifene, all of which appear to have different mechanisms of action, are able to function as ER agonists in the bone and the cardiovascular system (43). These compounds must possess a common functional activity that enables them to mimic estrogen in these targets. Although not presented in this study, we have been able to identify peptides that interact with the ER when activated by any ligand. The protein interaction surface implicated by this class of peptides may facilitate the interaction of the ER with specific transcriptional regulators within bone and the cardiovascular system. These findings, taken together with those presented here, indicate that the ER is a versatile transcription factor that manifests its biological action in different ways in different target cells.

The demonstration that GW5638 inhibits the growth of tamoxifen-refractory breast tumors is the most important finding of this study. If found to be effective when tested in the clinic, GW5638 will provide a second-line therapy for patients who present with tamoxifen-refractory ER-positive breast cancers. The benefits of second-line endocrine therapy for breast cancers is well established, but the most useful agents, aromatase inhibitors and Gonadotrophin Releasing hormone agonists, are not suitable for long-term use because of their negative impact on bone and other estrogen target organs (44). In addition, it has been shown that exposure of cultured breast cancer cells to aromatase inhibitors for extended periods leads to the development of sublines of cells that are hypersensitive to the mitogenic actions of estrogens (45). Clearly, because no single endocrine agent will be sufficient for the treatment of all ER-positive breast tumors, there is an unmet medical need for novel agents that target the estrogen signaling pathway in different ways.

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